

The University of Chicago

UTILIZATION OF GALACTOSE BY *ASPER-
GILLUS NIGER* AND *PENICIL-
LIUM GLAUCUM*

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1934

By
WORTHY H. HORR

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
PLANT PHYSIOLOGY
Vol. 11, No. 1, 1936

The University of Chicago

UTILIZATION OF GALACTOSE BY *ASPER-*
GILLUS NIGER AND *PENICIL-*
LIVM GLAUCUM

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1934

By
WORTHY H. HORR

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
PLANT PHYSIOLOGY
Vol. 11, No. 1, 1936



UTILIZATION OF GALACTOSE BY *ASPERGILLUS NIGER* AND *PENICILLIUM GLAUCUM*

W. H. H O R R
(WITH FIFTEEN FIGURES)

Literature

In working with the hexose sugar galactose, KNUDSON (12) found that in concentrations of 1-2 per cent. it was toxic to the seedlings of *Vicia villosa* and *Pisum sativum*. This toxicity was evidenced by a browning and killing of the roots. He also found that this toxicity was prevented by the addition of an equal concentration of dextrose to the solution. KNUDSON suggested that this might be brought about by a polymerization of the two sugars but he found no trace of lactose in the remaining solution. He also suggested that the toxicity might be due to oxidation products of galactose, and that glucose might prevent their absorption or modify their action. In a later paper (13) he found that galactose was toxic in concentrations as low as 0.0125 molecular solution, and that this toxicity was neutralized by saccharose, glucose, levulose, and lactose in 0.025 molecular concentration. He also found that mannose was toxic in 0.025 molecular solution and that this was neutralized by dextrose in an equal concentration. He states that mannose is not so toxic as galactose, and suggests that the dextrose might prevent the absorption of the galactose. Working with other sugars he found that sucrose and lactose caused a decided acceleration in the growth of timothy, and expressed the opinion that lactose is probably not assimilated by timothy; but he offered no explanation of this acceleration produced by the presence of lactose.

Similar effects of galactose on green seedlings were reported by HEINONEN (10), who repeated the work of KNUDSON and carried the studies further. She, too, found that dextrose neutralizes the toxicity of galactose, but that this neutralizing effect held only until the seedling used all of the dextrose in the solution and then there was a toxic action quite like that when pure galactose was supplied. It took several months, however, for the dextrose to be completely consumed and the toxicity to appear. She described the manifestations of this toxicity as bulbous enlargements of the root tips accompanied by a progressive brown discoloration of the epidermal and subepidermal tissues. She believed KNUDSON's suggestion that the dextrose prevented the absorption of galactose to be right, but did not present any sugar determinations on the used culture solutions to support this belief. She concluded that these were general non-specific responses, to be expected with toxins of various kinds, because of similar responses obtained by SOMMER and SOROKIN (28, 29) in their work on the deficiency of essential

elements. NIGHTINGALE *et al.* (21) reported a similar bulbous enlargement of root tips and a browning of the tissues followed by a general tissue sloughing in seedlings grown in a medium deficient in calcium. They did not state that these effects were due to a toxic action.

That other sugars may be toxic to green plants and their toxicity neutralized by the addition of a different sugar, is shown by the work of BRANNON (2), who found that fructose alone is toxic to peas and that its toxicity is neutralized by the presence of glucose. As he made no statement as to the nature of this toxicity, the effects cannot be compared with those noted by KNUDSON and HEINONEN.

The neutralizing effect of sugars on a non-sugar toxin is evidenced by the report of BOAS and MERKENSCHLAGER (1), who found that galactose is best and xylose second best, of the sugars which they used, for counteracting lime toxicity to *Lupinus luteus*.

One also finds that galactose may be utilizable to some degree, or be entirely unusable by some green plants other than those used by KNUDSON and HEINONEN. BURGE, WICKWIRE, ESTES, and WILLIAMS (3) found that *Spirogyra* and *Paramaecium* were able to utilize galactose, but not so well as they did dextrose and levulose. Similar results were obtained by ROBBINS (24) in his work on *Ceratodon purpureus*. This plant absorbed and used sucrose, levulose, maltose, dextrose, lactose, and galactose for growth and starch formation, but only a slight development was obtained when galactose and lactose were used as a source of carbon. These plants were unable to use mannite, glycerol, or starch. The experiments were carried on in darkness and no normal development of plants was obtained. ROACH (23) obtained a rapid growth of *Scenedesmus* on galactose, saccharose, glucose, and maltose, a moderate growth on levulose, but only a slight growth on mannite. With *Cystococcus* she obtained a rapid growth on mannite, glucose, fructose, and saccharose, but only a moderate growth on galactose and levulose.

According to the results of TERROINE and coworkers (30), peanut embryos were unable to utilize galactose, lactose, xylose, or arabinose, but they made a good uniform growth when supplied with dextrose, levulose, sucrose, or maltose. In this work there is no mention of any toxic effect from any of the non-assimilable sugars.

The carbohydrate-using non-green plants seem to be more tolerant toward different sugars. Their ability to utilize galactose as a source of carbon varies with the plant, but no cases of toxicity were found. PETERSON and FRED (22) report that *Lactobacillus pentoaceticus* was able to utilize galactose and dextrose with equal ease. Fifteen isolations of *Rhizoctonia* studied by MATSUMOTO (17) grew equally well on galactose and fructose, and in addition were able to use starch and amygdalin as carbon sources. *Plenodomus fuscomaculus* when grown by COONS (4) on M/20 dextrose and M/20 galac-

tose developed the same sized colonies, 2 mm. in diameter, and had the same form of fructification, oidia, at the end of two months. On raffinose, loose floccose colonies were developed which were larger than those produced on M/20 xylose. These were of solid compact growth, and the form of fructification was oidia. Lactose produced no growth at all.

KENDALL (11), working on the identification of carbohydrates by bacterial procedure, found only two of the bacteria he used (*Bacillus mesentericus* and *Vibrio* of Finkler and Prior) which were unable to ferment galactose.

With these varying data in mind the work described in this paper was undertaken. An attempt was made to determine: (1) whether or not galactose is toxic to some fungi as well as to some green plants; (2) if so what is the nature of this toxicity? (3) if there is a neutralization of toxicity by the presence of other sugars, how is this produced?

Methods

The stock cultures of the organisms used in these experiments, *Aspergillus niger* van Tiegh and *Penicillium glaucum* Link, were started from single spore cultures. The mineral nutrient medium used was COON'S "cheap synthetic solution" as described by YOUNG and BENNETT (32), to which 2 per cent. of sugar was added, except in some special tests. The solutions were sterilized in an Arnold steam sterilizer.

This concentration of sugar was used because it provided enough carbohydrate to prevent autolysis during the growth periods used, and according to NIETHAMMER'S (20) work with *Aspergillus niger* on sucrose it is well within the range of sugar tolerance of that organism.

The cultures were grown at 20° C. as that temperature was within the growth range of both organisms and there was a room available in which that temperature was maintained.

The rate of growth was taken from the dry weight of the mycelium formed. This was measured by filtering and drying the mycelium mats in Gooch crucibles. This method does not give an accurate measure because of the loss of spores through the filter. Porcelain filters which retained the spores were tried but they soon became clogged, and the rate of filtration was so reduced that they were impractical. Two or three flasks were used in each case and all the tests repeated several times.

Some linear growth rate measurements were made on agar in petri dishes, and on microscopic cover glasses. All pH readings were made with a Leeds and Northrup quinhydrone pH indicator. Before making the potentiometric determinations, the filtrate was boiled to remove the excess carbon dioxide.

The sugars used were prepared by the Pfanstiehl Chemical Company

and examined and declared pure by the United States Bureau of Standards, Department of Chemistry.

In setting up a series of culture flasks, a sufficient volume of mineral nutrient was made up to fill all flasks. From this enough dextrose and galactose solutions were made to make all pure solutions as well as the mixed solutions, thus insuring uniformity of the sugars.

All inoculations for a series were made at one time, using 1 cc. of a heavy spore suspension to insure a heavy uniform growth on each of the flasks, and each set within a series was filtered after the designated time had elapsed.

Experimentation

The strain of *Aspergillus niger* used in these experiments, while able to utilize galactose and mannose and develop both mycelium and spores, does not utilize either as readily as it does dextrose and levulose. With mixtures of galactose and dextrose, there was noted an acceleration of growth which caused, in some mixtures, a considerable increase in the dry weight of the mycelium produced. This acceleration in growth has always indicated two optimum concentrations of the individual sugars in the mixed solutions. The mixtures giving the optimum growth seem to vary with other conditions (fig. 1).

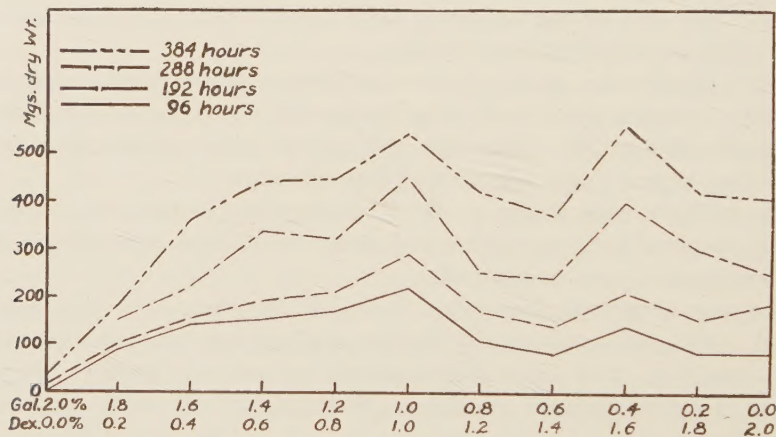


FIG. 1. *A. niger* grown at room temperature; 100 cc. of solution in each of two 125-cc. flasks.

In figure 2, where the organisms were given a larger surface area per unit of culture medium upon which to grow, the greatest acceleration occurred in the more dilute concentrations of galactose.

A similar growth reaction to mixtures of these sugars is exhibited by *Penicillium glaucum*, but as this organism utilizes the galactose much more readily than does *Aspergillus niger*, the acceleration is not so marked. It is

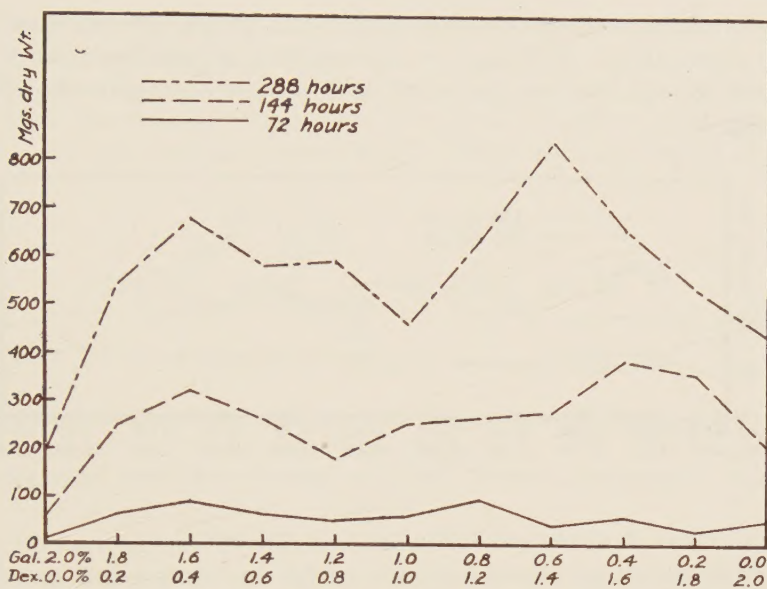


FIG. 2. *A. niger* grown at 20° C.; 100 cc. of solution in each of two 250-cc. flasks.

interesting to note that in nearly every case there is a greater amount of dry weight produced on the mixed sugars than is produced on the 2 per cent. dextrose solution (fig. 3).

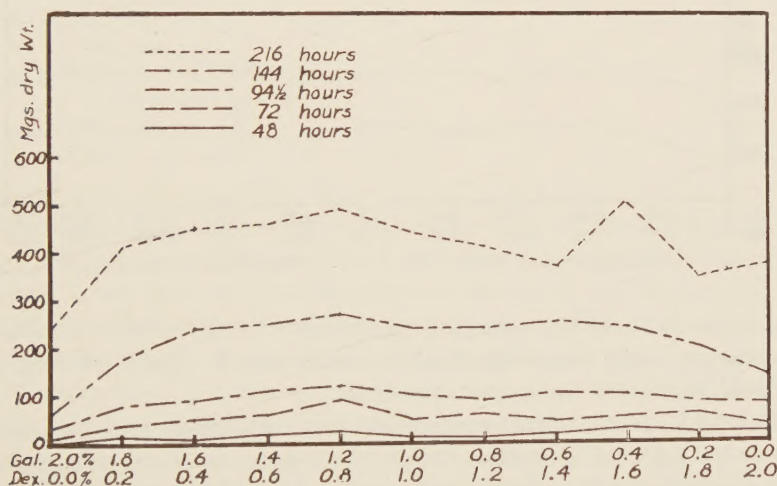


FIG. 3. *P. glaucum* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

The total sugar concentration in each solution was reduced to 0.5 per cent. to determine the growth rates on low sugar concentrations. Again the

mixtures of the two sugars usually produced a greater growth than either of the sugars alone. With the low concentration of galactose there is often a greater growth than was produced on the 2 per cent. galactose of other tests (fig. 4).

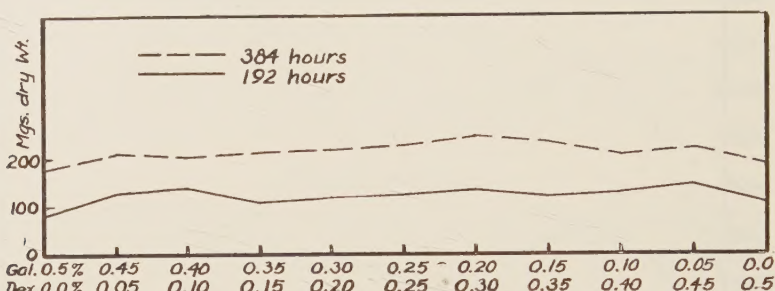


FIG. 4. *A. niger* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

Penicillium glaucum reacted similarly to *Aspergillus niger* on the dilute concentration, except that the growth on 0.5 per cent. galactose solution gave a smaller growth than was produced on the 2 per cent. galactose solution (fig. 5).

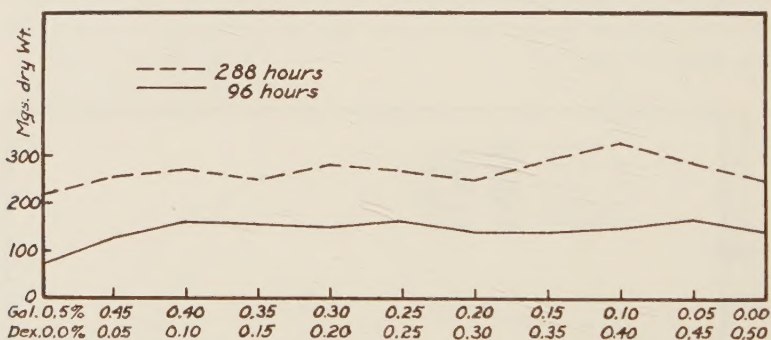


FIG. 5. *P. glaucum* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

In order to check the extent of acceleration in the solutions containing mixed sugars, flasks were made up as shown in table I. The 2 per cent. galactose flasks were made up merely as a check.

Each flask of pure sugars contained 100 cc. of solution and the tests were made on 1.8 per cent. galactose, 0.2 per cent. dextrose, 1 per cent. galactose, and 1 per cent. dextrose, alone and in mixtures. Where the solutions were mixed, four flasks, each containing 100 cc. of solution, were used to provide a growing surface and a total amount of sugar equal to that provided when each of the sugars was in a separate flask and two flasks used for each sugar.

Thus the surface exposed for growth and the total amounts of sugar available were equal in each comparison.¹

When the solution contained 1.8 per cent. galactose and 0.2 per cent. dextrose, there was produced three times as much dry weight as when the same amounts of sugars were in different flasks. With 1 per cent. of each sugar in



FIG. 6. *A. niger* grown 65 hours at 28° C. on 2% galactose.

the solution there was two and one-half times as much dry weight as when the sugars were used in different flasks (table I).

TABLE I

ASPERGILLUS NIGER, GROWN 168 HOURS AT 20° C.

SUGAR	NUMBER OF FLASKS	DRY WEIGHT
%		gm.
1. 2 galactose	2	0.0443
2. 1.8 galactose	2	0.0424
3. 1.0 galactose	2	0.0451
4. 1.0 dextrose	2	0.4110
5. 0.2 dextrose	2	0.1456
6. 1.8 galactose and 0.2 dextrose	4	0.5774
7. 1.0 galactose and 1.0 dextrose	4	1.1516
Adding nos. 3 and 4		0.4561
Adding nos. 2 and 5		0.1880

Linear growth was investigated on 2 per cent. galactose, 2 per cent. dextrose, and 1 per cent. galactose plus 1 per cent. dextrose in agar petri dish cultures. The measurements made were on the heavy visible portion of the mat without considering the microscopic mycelium which extended beyond. With 2 per cent. dextrose, and with 1 per cent. galactose plus 1 per cent. dextrose, the mats were of equal size and about twice the diameter of those on 2 per cent. galactose (table II). On agar, without sugar, the mats were of very meager thickness, but in all cases extended as far or farther than those on 2 per cent. dextrose (table III). Fruiting branches were very rare on the

¹ This does not give an exact measure of the amount of acceleration, as there is a greater area for the growth of the organism in the presence of the more usable sugar in the mixed solutions.

TABLE II
GROWTH OF *ASPERGILLUS NIGER*, DIAMETER OF MATS*

SUGAR	HOURS				
	52	76	100	124	148
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
2% galactose	4.95	6.65	6.65	11.2	16.1
2% dextrose	8.6	13.25	20.2	26.5	34.45
1% galactose plus 1% dextrose ...	8.0	12.62	19.62	26.75	34.75

* Each measurement is the average for five culture dishes.

medium which contained no sugar. It was apparent that there was a great difference in the thickness and density of the mats formed on the different sugars. These plates were inoculated with a small drop of spore suspension.

An attempt was made to correlate the density of the growth with the linear development of the mycelium. Agar petri dish cultures were again used and the total extent of the mat measured with a low power microscope. When no sugar was supplied there were again extensive but very meager mats formed, with no upright mycelium produced in five days. When galactose was used alone in the medium, the diameter of the mat was inversely proportional to the amount of sugar. When the sugars were mixed there was in both cases an acceleration. The relative thicknesses of the mats were estimated in order, without attempting an accurate measurement. Thus the figures in table IV give only relative thicknesses.

TABLE III
GROWTH OF *PENICILLIUM GLAUCUM*, DIAMETER OF MATS, PETRI DISH AGAR CULTURES, 20° C.

SUGAR	HOURS				
	52	76	100	124	148
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
2% galactose	5.95	9.10	12.15	15.15	17.95
2% dextrose	8.5	14.94	22.	29.12	35.19
1% galactose plus 1% dextrose ...	8.69	15.44	22.	28.75	35.12

The results given in table V show the growth rates of *Aspergillus niger* on galactose and dextrose and mixtures of these two sugars for 65 hours on agar coverglass cultures. In this test the germination of the spores is greatly delayed on the agar containing only galactose. There is also an abnormal growth on the galactose medium, as indicated by the crooked and much-

TABLE IV

GROWTH OF *ASPERGILLUS NIGER*, AGAR CULTURES AT 20° C. FOR FIVE DAYS

SUGAR	ORDER OF SPORULATION	DIAMETER OF MATS	THICKNESS*
2% galactose	5	mm. 2	7
1.8% galactose	9	14	8
1.0% galactose	7	20	6
1.0% galactose plus 1.0% dextrose	1	38	1
1.8% galactose plus 0.2% dextrose	4	35	4
0.2% dextrose	6	31	5
1.0% dextrose	3	34	3
2.0% dextrose ..	2	34	2
No sugar	8	30	9

* Order of relative thickness. Eight and nine, column 2, were practically equal, as were six and seven of column 4.

TABLE V

GERMINATION PERCENTAGE AND GROWTH (MM.) OF *ASPERGILLUS NIGER*, AGAR COVERGLASS CULTURES, 28° C.

SUGAR	HOURS						
	4	8	12	16	24	30	65
%							
2.0 galactose	0	0	0	0	25% 0.0375	90%†	95%
1.8 galactose plus 0.2 dextrose	50% 0.07	0.15	0.43	1.20†			
1.0 galactose plus 1.0 dextrose	50% 0.114	0.16	0.90				
1.0 galactose	0	0		5% 0.007*	15% 0.065	60%† 0.06	90% 0.10
0.2 dextrose	70% 0.0383	0.074	0.594	0.9†			
1.0 dextrose	65% 0.027	0.0825	0.465	0.67†			
2.0 dextrose	50% 0.025	0.1007	0.39	0.75†			

* Longest germ tube in the culture.

† Crooked, much branched mycelium.

‡ Increased germination shortened the average length of the germ tubes. Stopping of the measurements indicates that the mycelium was too long to be measured accurately.

branched mycelium shown in figure 6, as compared with figures 7 and 8. The growth on the dextrose and the dextrose plus galactose varies consid-



FIG. 7. *A. niger* grown 16 hours at 28° C. on 2% dextrose.

erably, with the greatest growth on the medium containing 1 per cent. of each sugar.

In mixing galactose and levulose, the growth rate is accelerated much as it is with galactose and dextrose. When mannose (which used alone as a carbohydrate supply also gives a very slight growth) was mixed with galactose there also resulted an acceleration comparable with that obtained with galactose and dextrose, or galactose and levulose. Levulose mixed with mannose, however, gave only a slight increase in growth over that obtained when levulose was used alone. When mannose was added to the solution containing galactose and levulose there was apparently no additional acceleration.

The dry weights of the mycelium mats in grams are given in figure 9, in

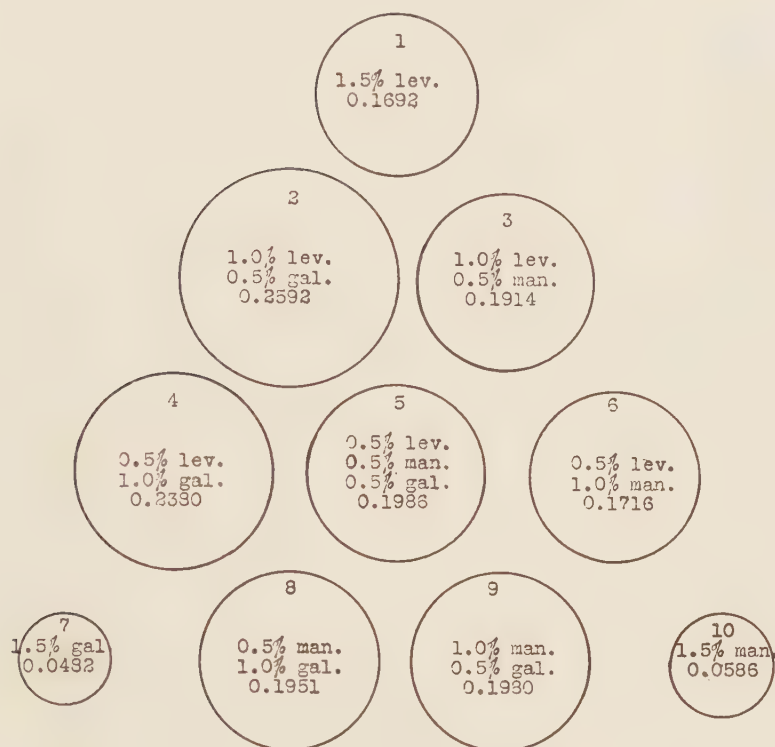
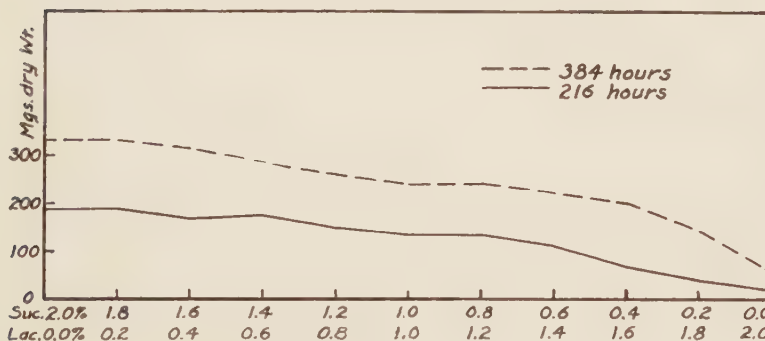


FIG. 8. *A. niger* grown 14 hours at 28° C. on 1% galactose and 1% dextrose.

which the area of each circle indicates the relative amount of dry weight produced.

The growth rates of *Aspergillus niger* and *Penicillium glaucum* on lactose and sucrose, pure and in mixtures, were investigated. In the curves of figures 10 and 11 there is no acceleration due to the presence of these two sugars in one culture solution. Reducing the amount of sucrose present does not greatly reduce the growth of the organisms, for the growth periods used, until the sucrose is reduced to 1 per cent. or less. There is then a more rapid drop in the amount of growth to a very low point on the 2 per cent. lactose solution.

A correlation was made between the dry matter formed and the increases in acidity during 48-hour periods. These preliminary experiments indicate that pure galactose produces the greatest acidity per gram of dry matter

FIG. 9. *A. niger* grown 14 days at 20° C. in three 150-cc. flasks.FIG. 10. *P. glaucum* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

formed, galactose plus dextrose produces the least, and pure dextrose is intermediate in the production of acid. The total acidity in the pure galactose is less than that produced on the pure dextrose or on the dextrose plus galactose, indicating that it must be within the range for favorable growth of these organisms (figs. 12-14).

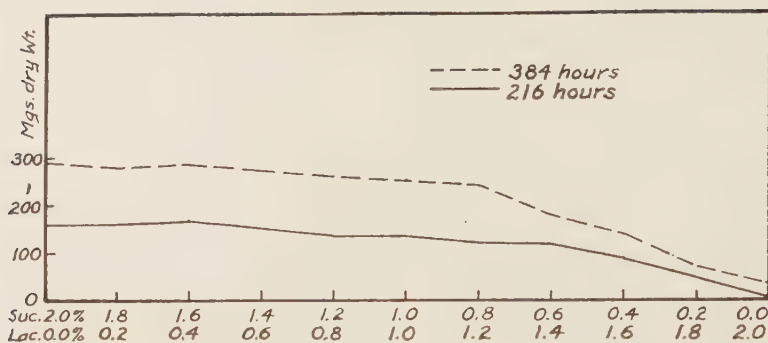


FIG. 11. *A. niger* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

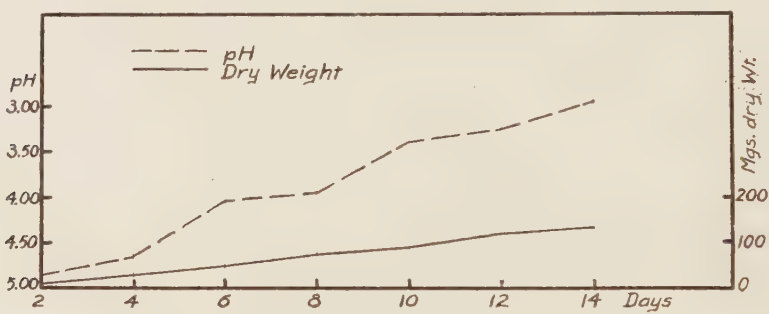


FIG. 12. 2% galactose.

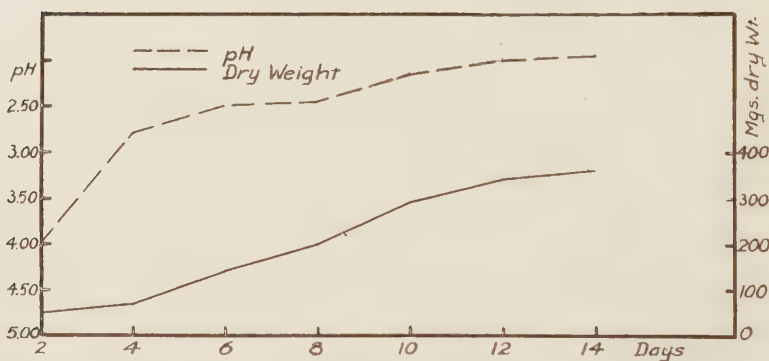


FIG. 13. 2% dextrose.

Comparisons were made with cultures grown on solutions sterilized in the Arnold steam sterilizer and those grown on cultures sterilized by filtering through porcelain filters. The data presented in figure 15 eliminate the possibility of there being any significant changes in the mixed sugars during the process of heat sterilization.

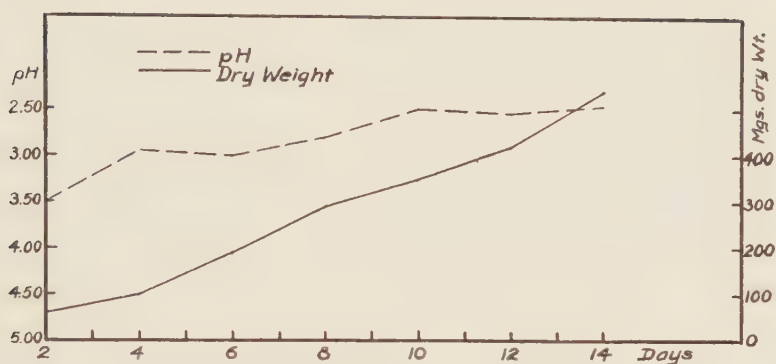


FIG. 14. 1% galactose and 1% dextrose.

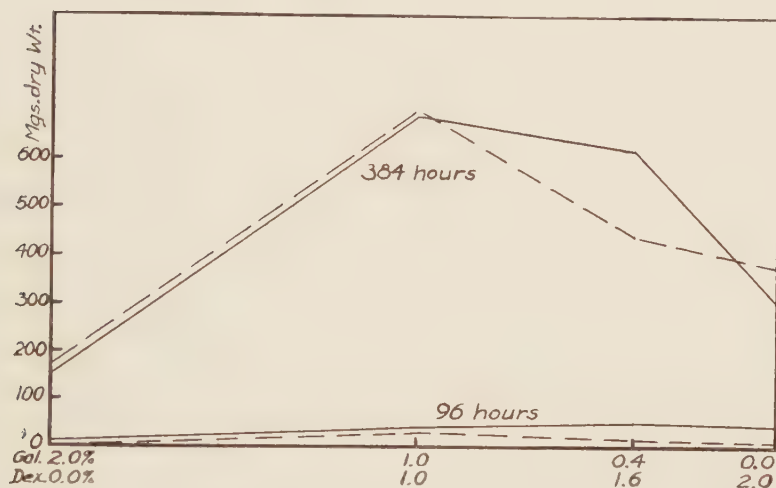


FIG. 15. ——— sterilized in Arnold; - - - - - sterilized by filtration.

Discussion

The frequent occurrence in plants of compounds which upon hydrolysis yield galactose would tend to imply that that sugar must play some part in the normal metabolism of those plants. LIPPMAN (16) found a thin coat of galactose on the surface of frozen ivy fruits. It is not positively known that this did not result from hydrolysis of a glucoside. CZAPEK (5) states that it is questionable whether galactose ever occurs free in plants.

Among the compounds which upon hydrolysis yield galactose are glucosides and galactans. The first named are probably split up by enzymes, but GORTNER (8) states that "insofar as is known, there are no known specific enzymes which will hydrolyze galactans." On the other hand, HAAS and HILL (9) state that "the galactans found in the cotyledons of many plants are used as reserve foods."

MAXIMOV (18) states: "Of the sugars, only a certain group is readily transformed by the lower plants, namely, glucose, d-fructose, and d-mannose. All of these sugars have a related configuration of the atomic groups, and, with hydroxyl migration they exhibit the same enolic form. Galactose showing a somewhat different configuration is utilized with greater difficulty."

In this work glucose and levulose were found to be good sources of carbon for *Aspergillus niger* and *Penicillium glaucum*, while mannose and galactose were very poor sources. KNUDSON (13) found that mannose acted more like galactose in that it was toxic to some green plants and that this toxicity was neutralized by the addition of glucose or sucrose.

KENDALL (11) found that dextrose is the only sugar which is fermented by all sugar-fermenting organisms.

EULER, LAURIN, and PATTERSON (6) found a top yeast which was able to adapt itself to galactose as a source of carbon and in so doing increased its ability to ferment galactose fifty times faster than the initial rate. The yeast reached the point where it fermented galactose six and one-half times as fast as it could ferment cane sugar. SÖHNGEN and COOLHAAS (27), working with the yeast *Saccharomyces cerevisiae*, found that it developed a new enzyme, galactose-zymase, which was capable of fermenting galactose. This enzyme disappeared when the galactose was removed as a source of carbon.

Aspergillus niger and *Penicillium glaucum* have shown an apparent increase in their ability to utilize galactose after being supplied this sugar as a source of carbon. However, no quantitative determinations have been made to test this increased ability. From this, and the above cited works, one would conclude that many lower plants which utilize galactose with difficulty are capable of readily utilizing it as a source of carbon if given an opportunity to develop and operate the proper machinery for such utilization.

It is shown by this work that galactose alone is not only a poor source of carbon for *A. niger*, but that it slows up spore germination, retards growth of the mycelium, and also causes abnormal growth. This is shown in table V where there are visible signs of germination of *A. niger* spores in 16 hours on 1 per cent. galactose, but 24 hours were required on a 2 per cent. galactose solution. On dextrose, dextrose plus galactose, and on agar alone there was at least 50 per cent. germination within the first four hours. When the same mineral nutrients and agar without sugar are supplied we find the development of mycelium threads much longer than when galactose has been added, thus indicating that galactose under these conditions actually retards the growth of *A. niger* on a medium where it would normally produce a limited amount of mycelium. Figure 6 shows that on agar containing only galactose as a source of carbon the mycelium threads are much branched and very crooked as compared with those on 1 per cent. dextrose or 1 per cent. dextrose plus 1 per cent. galactose, as is shown in figures 7 and 8.

KUNKEL (15) states that toxicity retards germination and growth of fungi. SCHELLING (25) claims that vitamin B preparations stimulate *A. niger* to abnormal development like that caused by toxins, namely, short, blunt, irregularly shaped branches. WATERMAN (31) finds that mutation of *Penicillium glaucum* is brought about in cultures which retard growth. He lists lactose, galactose, boric acid, and all narcotics as substances which check the growth of *P. glaucum* and cause mutation. This mutation appears after about 14 months. He also found that *A. niger* mutates from the effects of:

- A. Poisons such as copper sulphate and benzoic acid.
- B. Narcotics such as paraoxybenzoic acid and salicylic acid.
- C. (1) Culture media with galactose or polysaccharides containing the galactose group as a carbon source; (2) culture media with glutaric acid, tartaric acid, antitartaric acid, and rhamnose.

This mutation consists largely of a checking of growth, a loss of color, and a reduction in the number of spores produced.

If the retardation of germination and growth and the development of abnormal mycelium are accepted criteria for toxicity, then, according to the present data, galactose when used alone as a source of carbon is toxic to *A. niger* and *P. glaucum*. However, I would hesitate to accept these results as indications of toxicity. Would it not be possible for galactose to act as a growth retarding substance, on *A. niger* and *P. glaucum*, without necessarily being toxic? This might be true of other so-called toxins which do not cause any actual destruction of cells, as was reported by KNUDSON (12) and HEINONEN (10).

It is evident from the data of BOAS and MERKENSCHLAGER (1), BURGE *et al.* (3), ROBBINS (24), and ROACH (23) that galactose is not toxic to all green plants. Also, the literature indicates that some non-green plants are able to utilize galactose as well as or even better than they can other sugars.

In the work of KNUDSON (12) and HEINONEN (10) it was found that the addition of some other sugars neutralized the toxicity of galactose toward the green plants they used. In my work it is found that when dextrose, levulose, or mannose is added to galactose, there is a decided acceleration in the growth of *A. niger* and *P. glaucum*, as indicated by the weight of dry matter formed. This is similar to the results of KNUDSON (14), who found that when timothy seedlings were grown on a medium containing lactose and sucrose there was a decided acceleration in growth rate. When KNUDSON mixed galactose and mannose, both of which he found to be toxic to the plants he worked with, there was no neutralization of the toxicity; whereas in my work there was a decided acceleration in growth when these two were mixed. The degree of this acceleration is determined by the percentage of

each sugar in the individual mixtures used and is apparently modified by other factors. In each set of culture flasks set up as in figures 1, 2, and 3, there has always been two optimum solutions. These remain relatively constant for the series set up from a given stock solution, but there are some variations between the sets set up from different stock solutions. The cause of this variation has not been determined. Probably if the average were taken from enough sets of cultures made up from different stock solutions, there would be only one growth peak which would cover several of the mixtures.

This acceleration in growth must be due to the presence of the galactose, since there is no appreciable acceleration when mannose and levulose are mixed as in numbers 3 and 6, figure 9. This is also true in figures 10 and 11. The last two examples correspond to the work of SOBOTKA and REINER (26), in which they report that the rate of fermentation of mixed sugars is barely greater than that of the fastest fermenting component. This cannot be the case when galactose is mixed with glucose, levulose, or mannose and used as a source of carbon for *A. niger* and *P. glaucum* because of the acceleration obtained from the mixtures of sugars.

This acceleration in growth may produce three times as much dry weight on two sugars mixed as the sum of the dry material produced on the same sugars used separately, the total amount of sugars and growing surfaces being equal in each case. This is seen in comparing the total weight produced by numbers 2 and 5 with number 6, and that of 3 and 4 with number 7, of table I.

Table V shows that during the early growth period, the first 16 hours, there is an acceleration in the rate of elongation of the mycelium when there is galactose mixed with dextrose, over that on either of the sugars alone.

If one is to accept MAXIMOV'S (19) statement that "of the great variety of organic compounds of different degree of availability plants will always utilize the more available compounds and only then begin to use the less assimilable ones," this growth then must all be at the expense of the dextrose, mannose, or levulose present in the mixed solutions. If this is true, then we find galactose acting exactly opposite from what it does when used alone. KNUDSON (14) suggested that there might be a polymerization of the galactose and dextrose to form lactose and thus neutralize the toxic action of galactose. Referring to figures 10 and 11, it is seen that such could not be the case here because of the very small amount of growth on lactose. KNUDSON also suggested that dextrose might prevent the absorption of galactose. That would not apply to this work, because with the addition of galactose to dextrose, levulose, or mannose there is an acceleration. This acceleration surely would not occur unless the galactose in some way came into contact with the protoplasm of the growing cells. Another suggestion by KNUDSON (12) is

that the oxidation products of galactose may be toxic to green plants. This is in accord with the idea of FUNKE (7) that galactose and mannose do not prevent enzyme production but that their metabolic products do. This, too, probably would not apply to the results here presented because of the inhibition of germination and growth from the time of inoculation.

There is no clue to this growth acceleration found in figures 12, 13, and 14, where the dry weight and the increase in acidity are compared.

This leaves unsolved the question as to the action of any one sugar when it neutralizes the toxicity of another sugar, or when galactose causes an acceleration in the growth of *A. niger* and *P. glaucum* when it is mixed with dextrose, levulose, or mannose in the culture solution.

Summary

1. Galactose and mannose are poor sources of carbon for *Aspergillus niger* and *Penicillium glaucum*.

2. Galactose, when used alone as a source of carbon, decreases the rate of spore germination and mycelium development and causes abnormal mycelium development.

3. Galactose, when mixed with dextrose, levulose, or mannose, causes an acceleration in growth. This is not caused by mannose, which is also a poor source of carbon for these organisms.

4. The addition of lactose to a culture solution containing sucrose has little if any effect upon the growth rate.

5. Preliminary correlations on the amount of dry matter formed and the acidity developed do not indicate that the acidity of the solution plays any important part in the acceleration of growth.

6. The data hold for the strains of *A. niger* and *P. glaucum* used; other organisms or other strains of these organisms may react entirely differently.

The writer wishes to express his appreciation to Dr. CHARLES A. SHULL for the many helpful suggestions and criticisms offered throughout the progress of this work. He is also grateful to The Elizabeth Thompson Science Fund for a grant for the purchase of sugars, and to The Graduate Research Fund of the University of Kansas for grants for the purchase of apparatus and supplies.

UNIVERSITY OF KANSAS
LAWRENCE, KANSAS

LITERATURE CITED

1. BOAS, F., and MERKENSCHLAGER, F. Über die Wirkung spezifischer Zuckerarten bei höheren Pflanzen. Ber. deutsch. bot. Ges. **41**: 187-190. 1923.

2. BRANNON, J. M. Influence of certain sugars on higher plants. Bot. Gaz. **75**: 370-389. 1923.
3. BURGE, W. E., WICKWIRE, G. C., ESTES, A. M., and WILLIAMS, MAUDE. Stimulating effect of amino acids on sugar metabolism of plant and animal cells. Bot. Gaz. **85**: 344-347. 1928.
4. COONS, G. H. Factors involved in the growth and the pycnidium formation of *Plenodomus fuscomaculans*. Jour. Agr. Res. **5**: 713-769. 1916.
5. CZAPEK, FRIEDRICH. Biochemie der Pflanzen. Dritte Auflage. Vol. I. p. 265. G. Fischer. 1922-25.
6. EULER, H. V., LAURIN, I., and PETTERSSON, A. Anpassung einer Oberhefe an das Gärsubstrat Galaktose. Biochem. Zeitschr. **114**: 277-291. 1920.
7. FUNKE, F. L. Researches on the formation of diastase by *Aspergillus niger*. II. Rec. Trav. Bot. Neerl. **23**: 200-244. 1926.
8. GORTNER, R. A. Outlines of biochemistry. p. 559. John Wiley & Sons. 1920.
9. HAAS, R., and HILL, T. G. An introduction to the chemistry of plant products. Vol. III. 3rd ed. p. 139. Longmans Green & Co. 1921.
10. HEINONEN, JUNETTA C. Unpublished thesis, University of Chicago. Cited in Biol. Abst. **VII**. 12894. 1933.
11. KENDALL, A. J. Carbohydrate identification by bacterial procedure. Jour. Infect. Diseases **32**: 362-368. 1923.
12. KNUDSON, LEWIS. Toxicity of galactose for certain of the higher plants. Ann. Missouri Bot. Gard. **2**: 659-666. 1915.
13. —————. Influence of certain carbohydrates on green plants. Cornell Agr. Exp. Sta. Bull. **9**. 1916.
14. —————. The toxicity of galactose and mannose for green plants and the antagonistic action of other sugars toward these. Amer. Jour. Bot. **4**: 430-437. 1917.
15. KUNKEL, O. The influence of starch, peptone, and sugar on the toxicity various nitrates to *Monilia setophila*. Bull. Torr. Bot. Club **40**: 625-639. 1913.
16. LIPPMAN, E. O. Edenda. **43**: 3611. 1910.
17. MATSUMOTO, TAKASHI. Morphology and physiology of fifteen isolations of *Rhizoctonia*. Ann. Missouri Bot. Gard. **8**: 1-62. 1921.
18. MAXIMOV, N. A. Textbook of plant physiology. 1st ed. Ed. by A. E. MURNEEK and R. B. HARVEY. p. 233. McGraw-Hill Book Co. 1930.
19. —————. Textbook of plant physiology. 1st ed. Ed. by A. E.

- MURNEEK and R. B. HARVEY. p. 234. McGraw-Hill Book Co. 1930.
20. NIETHAMMER, A. Über das Gesetz von Minimum bei Pilzkulturen. *Biochem. Zeitschr.* **165**: 168-195. 1925.
 21. NIGHTINGALE, G. T., ADDOMS, RUTH M., ROBBINS, W. R., and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. *Plant Physiol.* **6**: 605-630. 1931.
 22. PETERSON, W. H., and FRED, E. B. Fermentation of glucose, galactose, and mannose, by *Lactobacillus pentoaceticus* n. sp. *Jour. Biol. Chem.* **42**: 273-287. 1920.
 23. ROACH, B. MURIEL. On the relation of certain soil algae to some soluble carbon compounds. *Ann. Bot.* **40**: 149-203. 1926.
 24. ROBBINS, WM. J. Direct assimilation of organic carbon by *Ceratodon pupureus*. *Bot. Gaz.* **65**: 543-551. 1918.
 25. SCHELLING, N. J. Growth stimulation of *Aspergillus niger* by a vitamin B preparation. *Bull. Torr. Bot. Club* **52**: 291-310. 1925.
 26. SOBOTKA, H., and REINER, M. Alcoholic fermentation of glucose, fructose, and mannose mixtures. *Biochem. Jour.* **24**: 926-931. 1930.
 27. SÖHNGEN, N. L., and COOLHAAS, C. The fermentation of galactose by *Saccharomyces cerevisiae*. *Jour. Bact.* **9**: 131-141. 1924.
 28. SOMMER, A. L., and SOROKIN, HELEN. Effects of the absence of boron and of some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. *Plant Physiol.* **3**: 237-261. 1928.
 29. SOROKIN, HELEN, and SOMMER, A. L. Changes in the cells and tissues of root tips induced by the absence of calcium. *Amer. Jour. Bot.* **16**: 23-39. 1929.
 30. TERROINE, EMILE, TRAUTMAN, F. S., BENNET, R., and JACQUET, R. L'énergie de croissance. IV. Le rendement énergétique des divers glucides dans la croissance des végétaux supérieurs. *Bull. Soc. Chim. Biol.* **7**: 461-473. 1925.
 31. WATERMAN, H. J. Mutation bei *Penicillium glaucum* und *Aspergillus niger*. *Zeitschr. Gärungsphysiologie* **3**: 1-15. 1913.
 32. YOUNG, H. C., and BENNETT, C. W. Growth of some parasitic fungi on synthetic culture media. *Amer. Jour. Bot.* **9**: 459-469. 1922.

